Preventing Engrailed-1 activation in fibroblasts yields wound regeneration without scarring.

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Public Summary:
Regeneration without scarring Wounds in adult mammals typically heal by forming fibrotic scars. Mascharak et al. found that a specific population of skin fibroblasts (Engrailed-1 lineage–negative fibroblasts) activate expression of Engrailed-1 and turn on profibrotic cellular programs in response to local tissue mechanics in wounds (see the Perspective by Konieczny and Naik). When mechanical signaling was inhibited in these cells (using either genetic deletion or small-molecule inhibition), skin wounds in mice no longer formed scars but instead healed by regeneration, restoring skin with normal hair follicles and glands, extracellular matrix, and mechanical strength. Science, this issue p. eaba2374; see also p. 346 Mechanical tension on wounds provokes dermal fibroblasts to produce scars, presenting a therapeutic target for tissue regeneration. INTRODUCTION Skin wounds generally heal by scarring, a fibrotic process mediated by the Engrailed-1 (En1) fibroblast lineage. Scars differ from normal unwounded skin in three ways: (i) They lack hair follicles, sebaceous glands, and other dermal appendages; (ii) they contain dense, parallel extracellular matrix fibers rather than the “basket-weave” pattern of uninjured skin; and (iii) as a result of this altered matrix structure, they lack skin’s normal flexibility and strength. A successful scar therapy would address these three differences by promoting regrowth of dermal appendages, reestablishment of normal matrix ultrastructure, and restoration of mechanical robustness. However, little is known about the cellular and molecular mechanisms blocking a regenerative healing response in postnatal skin, or whether these mechanisms can be bypassed by modulating specific fibroblast lineages. RATIONALE We asked whether scarring fibroblasts are derived purely from expansion of existing En1 lineage–positive fibroblasts present in unwounded skin, or whether En1 scar fibroblasts could arise de novo by activation of En1 expression in postnatal, En1 lineage–negative fibroblasts within the wound niche. We used fibroblast transplantation as well as transgenic mouse models to trace En1 expression in a spatiotemporally defined fashion. Next, we studied fibroblast responses to mechanical forces in vitro and in vivo to establish a mechanotransduction mechanism linking skin tension to postnatal En1 expression. Finally, we used chemical (verteporfin) and transgenic inhibition of mechanotransduction signaling [diphtheria toxin ablation of En1-expressing fibroblasts, floxed Yes-associated protein (YAP) knockout] to modulate En1 expression during wound healing. Experimental wounds were compared to unwounded skin and scars (phosphate-buffered saline control) by RNA sequencing, quantitative histopathological comparison (using a custom image-processing algorithm), and mechanical strength testing. RESULTS Fibroblast transplantation and lineage-tracing studies reveal that En1 lineage–negative fibroblasts (ENFs) of the reticular (deep) dermis activate En1 in the wound environment, generating ~40 to 50% of scar fibroblasts. This phenomenon depends on mechanical cues: ENFs cultured on soft substrates or treated with chemical inhibitors of mechanical signaling proteins (e.g., YAP) do not activate En1. Comparison of ENFs with En1-expressing and En1 knockdown (short hairpin RNA) fibroblasts by RNA sequencing suggests that En1 regulates a wide array of genes related to skin fibrosis. In healing wounds, YAP inhibition by verteporfin blocks En1 activation and promotes ENF-mediated repair, yielding skin regeneration in 30 days with recovery of functional hair follicles and sebaceous glands. Quantitative comparison of scars and regenerated skin shows that YAP inhibition induces recovery of normal dermal ultrastructure, which in turn confers restoration of normal mechanical breaking strength. Diphtheria toxin–mediated ablation of postnatal En1-expressing fibroblasts and fibroblast-targeted transgenic YAP knockout similarly promoted recovery of normal skin structures, which suggests that modulation of En1 activation, whether direct or indirect, can yield wound regeneration. CONCLUSION By delineating how physical stimuli provoke ENFs to contribute to fibrosis, we identify YAP and En1 as possible molecular targets to prevent scarring. Furthermore, we have shown that inhibition of YAP signaling prevents En1 activation during wound healing, thus encouraging ENF-mediated wound repair without fibrosis and with regeneration of secondary skin elements (hair follicles, sebaceous glands). Our findings suggest that residual ENFs in postnatal mammalian skin retain a capacity for skin

regeneration if the mechanically driven propensity for fibrosis can be blocked. We have demonstrated fully regenerative skin healing in a postnatal mammal that normally scars; this finding has translational implications for the tens of millions of patients each year who develop scars and other fibroses. Engrailed-1 activation in skin fibroblasts drives scarring. After injury, a subset of dermal fibroblasts activates Engrailed-1 (En1) to contribute to scarring (left). Inhibiting postnatal En1 activation, either directly (by ablating En1-activating cells) or indirectly (by blocking mechanical signaling with verteporfin), promotes skin regeneration by En1 lineage–negative fibroblasts, with full recovery of normal hair follicles, glands, matrix ultrastructure, and mechanical strength. Green cells, En1 lineage–positive fibroblasts; red cells, En1 lineage–negative fibroblasts. Skin scarring, the end result of adult wound healing, is detrimental to tissue form and function. Engrailed-1 lineage–positive fibroblasts (EPFs) are known to function in scarring, but Engrailed-1 lineage–negative fibroblasts (ENFs) remain poorly characterized. Using cell transplantation and transgenic mouse models, we identified a dermal ENF subpopulation that gives rise to postnatally derived EPFs by activating Engrailed-1 expression during adult wound healing. By studying ENF responses to substrate mechanics, we found that mechanical tension drives Engrailed-1 activation via canonical mechanotransduction signaling. Finally, we showed that blocking mechanotransduction signaling with either verteporfin, an inhibitor of Yes-associated protein (YAP), or fibroblast-specific transgenic YAP knockout prevents Engrailed-1 activation and promotes wound regeneration by ENFs, with recovery of skin appendages, ultrastructure, and mechanical strength. This finding suggests that there are two possible outcomes to postnatal wound healing: a fibrotic response (EPF-mediated) and a regenerative response (ENF-mediated).

Scientific Abstract:
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